A novel single-stranded DNA detection method based on organic semiconductor heterojunction

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ABSTRACT: We demonstrate a novel DNA detection method with low-cost and disposable advantages by utilizing F₁₆CuPc/CuPc **planar** organic heterojunction device. Single-stranded DNA (ssDNA) molecules have been well immobilized on the surface of CuPc film observed by atomic force microscopy, producing an obvious electrical response of the device. The conductivity of the organic heterojunction film was significantly increased by ssDNA immobilization because ssDNA molecules brought additional positive charges at heterojunction interface. Furthermore, the thickness dependence of CuPc upper layer on the electrical response was studied to optimize the sensitivity. This study will be helpful for the development of organic heterojunction based biosensors.

KEYWORDS: Organic heterojunction; charge accumulation; DNA detection; Biosensors; Copper phthalocyanine

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1. Introduction

The study of deoxyribonucleic acid (DNA) detection technologies has experienced a rapid development in the past decade. Traditional DNA biosensor makes use of fluorescence-labeled detection techniques to determine DNA hybridization. The labeled techniques are complicated and costly because they need large optical systems and analysis procedure [1, 2]. Recently, label-free techniques have been studied with much interest because of removing the labeling step, including surface plasmon resonance [3], scanning Kelvin probe microscopy [4], quartz crystal microcantilever [5], and genetic field-effect transistor (FET) [6]. Among all the label-free techniques, the use of electronic devices for detecting biological molecules is very promising because of its simplicity in signal processing and convenience in electronic integration [7, 8]. Moreover, electronic device such as FET fabricated by organic semiconductors is an excellent candidate for the application as low-cost and disposable sensors for its low-cost and easy fabrication process [8, 9].

Organic semiconductors have been extensively studied for several decades. Much progress has been made in the discovery and optimization of electrical, optical, structural and mechanical properties of various organic semiconductors for device applications. In this paper, we give a first evidence of an organic semiconductor heterojunction device with planar structure to detect the presence of single-strand DNA (ssDNA) biomolecules. The device structure is similar to thin-film transistor (TFT) but it has no gate electrode and dielectric layer, and the channel in this device is intrinsically conductive [10]. It has been reported that the interface in organic heterojunction generally exhibits an enhanced electrical conductivity because large amounts of carriers can be accumulated at the heterojunction interface by charge transfer [11, 12]. Moreover, these carriers can be easily affected by external influences, including light [13, 14], gas [15, 16] and biomolecules. For the case of DNA detection, it is well known that ssDNA is an important probe for detecting the target DNA. Therefore, it is very necessary for us to comprehensive study about the ssDNA immobilization and the corresponding electrical response in the planar organic heterojunction

devices. This study will be helpful for the development of **planar** organic heterojunction based biosensors.

2. Experiment

All the devices were fabricated on glass substrate with bottom-contact structure. The core of the device is an organic heterojunction, which is based on n-type copper-hexadecafluoro-phthalocyanine ($F_{16}CuPc$) and p-type copper phthalocyanine ($CuPc$). The fabrication process of the **planar** organic heterojunction device used in the experiment will be described in detail below. First 30nm drain-source electrodes were deposited on glass substrate using gold by vacuum thermal evaporation and patterned by means of photolithography. The fabricated devices have a channel length of 30 μm and electrode width of 1mm. Then 15 nm $F_{16}CuPc$ thin films were deposited on the channels. Subsequently, 15nm $CuPc$ thin films were deposited on $F_{16}CuPc$ to form organic heterojunction. All organic semiconductors were deposited in a fixed rate of 1 $\text{\AA}/s$ under a pressure of $2\sim5\times10^4$ Pa. The substrate was kept at 150 $^{\circ}C$ during deposition. The thickness and deposition rate were recorded by quartz oscillator. The organic semiconductor materials were purchased from Sigma-Aldrich without further purification. All electrical measurements were performed in a dark room and in ambient by using Agilent 4155C. All surface morphology of the organic films was characterized by SPI4000 atomic force microscopy (Seiko Instruments Inc.) in tapping mod

In current experiment, physical adsorption was used to immobilize the ssDNA on the $CuPc$ film surface. The operation process was following a procedure reported previously [2]. The ssDNA molecules 5'-TGC AGT TTT CCA GCA ATG AG-3' (Sangon Shanghai Ltd.) were dissolved in phosphate buffer solution (PBS) with concentration of 50 $\mu g/ml$ and PH of 7.4. The devices were first stored in air over night to get stable. Then PBS with 50 $\mu g/ml$ ssDNA was dropped onto the device channel by micro pipette and kept in a nearly airtight container for 1h to immobilize ssDNA on $CuPc$ adequately. Subsequently the samples **were** rinsed by DI water for 2min and the residual water **in** the samples was removed by an air gun with high pressure, afterwards the samples were stored in nitrogen ambient at room temperature for a few hours to dry the samples and eliminate the

oxygen/moisture effects. And then, we prepared a series of solutions with different concentrations to further study the ssDNA molecules immobilization on the planar organic heterojunction devices (0.5, 5, 10, 30, 50 $\mu\text{g/ml}$). For well evaluating the air stability of the planar organic heterojunction devices, some samples were measured for several times during a period of about one week (173 hours).

3. Results and Discussion

The electrical characteristics of the planar heterojunction device before and after ssDNA immobilization are shown in Fig. 1(a) and (b). Obvious increase in the current has been found when ssDNA was immobilized on the surface of CuPc implies that ssDNA molecules have an apparent electrical response on the device. For the device without ssDNA, same number of electrons and holes are accumulated on both sides of the heterojunction interface for being neutral in the whole heterojunction thin film [11]. The equilibrium state is broken by the immobilization of ssDNA molecules which have negatively charged phosphate groups on their backbone. Therefore, lots of additional positive charges in CuPc are induced by the negative charges in ssDNA molecules [2], resulting in an increased hole density in CuPc layer, and hence, the conductivity of the heterojunction device is improved. In addition, all devices exhibit typical linear characteristics indicating that good ohmic contacts were formed between the organic semiconductors and Au electrodes. Therefore, a constant resistance of each device can be easily extracted from the I-V curves. The calculated resistance of the device without ssDNA is 21.72 $\text{M}\Omega$ and it reduces to 16.29 $\text{M}\Omega$ after ssDNA immobilization.

For verifying the immobilization of ssDNA molecules on CuPc surface, the morphology of CuPc thin films before and after ssDNA immobilization were characterized by SPI4000 atomic force microscopy (Seiko Instruments Inc.) in tapping mode. As seen in Fig. 2 (a) for the original sample, the CuPc thin film exhibits typical disorder crystals with noodles-like shape. In contrast to the original sample, the CuPc thin film with ssDNA molecules displays a completely different morphology with indistinct CuPc crystals, which is shown in Fig. 2 (b). By the amplification of this image, an apparent stripe-like substance with about 10-15nm wide can be clearly observed that are surely ssDNA molecules gathered on the surface of CuPc by physical absorption, and the size and

shape of the ssDNA molecules is in basically accordance with the previous reports [2]. The results indicate that ssDNA molecules can be well immobilized on CuPc surface. Moreover, the crystal boundaries of CuPc thin film could be filled by ssDNA molecules. As a consequence, the root mean square roughness of CuPc thin film reduced from 3.089 to 2.569 nm after ssDNA immobilization.

Moreover, transmission line model (TLM) patterns were used to evaluate the contact resistance and the real bulk resistance of the organic heterojunction film. The TLM pads were equivalent to the drain-source electrodes and the spacing between the pads was equivalent to the channel length, which were 30, 50, 70 and 90 μm . Fig. 3(a) shows the current of the devices measured at 50V under different channel length before and after ssDNA immobilization. All devices exhibit increased current when ssDNA molecules are immobilized. Moreover, the increase rate is different for devices with different channel length. Hence, we define the current increase rate as the **electrical response** of this device:

$$S = \left(\frac{\Delta I}{I_o} \right) \times 100\% \quad (1)$$

where $\Delta I = I_{\text{DNA}} - I_o$; I_o is the original current value, and I_{DNA} is the current of device after ssDNA immobilization. The **current increase rate** is 29.3 % while the channel length is 30 μm . But it increased to 34.2 % with increasing the channel length to 90 μm , indicating that longer channel length results in higher sensitivity for ssDNA detection.

Though the total resistance of device can be calculated from the I-V curves, it should be noted that the total resistance also contains the contact resistance between electrodes and semiconductors. The total resistances of the heterojunction devices under different channel length are shown in Fig. 3(b). It is obvious that the total resistances of all devices are reduced after the immobilization of ssDNA because they are inversely proportional to the current. The **channel resistance (R_{ch})** can be obtained from the following equation:

$$R_{\text{tot}} = R_{\text{ch}} + 2R_c \quad (2)$$

Due to the same interface contact nature between Au and organic semiconductors, the contact resistance (R_c) is same for all devices with different channel length. At the same time, R_{ch} is proportional to the channel length. R_{tot} is the total resistance. The calculated channel resistance per unit length for the heterojunction without ssDNA is $0.641 \text{ M}\Omega/\mu\text{m}$, while it reduces to $0.479 \text{ M}\Omega/\mu\text{m}$ after the immobilization of ssDNA. In addition, the average thin film resistivity of the heterojunction can be obtained according to following equation:

$$R_{ch} = \frac{\rho l}{s} \quad (3)$$

Where l is the channel length and s is the cross-sectional area of the heterojunction. The thickness of the heterojunction is approximately 30nm (by adding the thickness of CuPc and $F_{16}\text{CuPc}$) and the width of the electrodes is 1mm, as described in the experiment part. The calculated average thin film resistivity of the organic heterojunction film before and after ssDNA immobilization is $1.92 \times 10^3 \Omega \cdot \text{cm}$ and $1.43 \times 10^3 \Omega \cdot \text{cm}$, respectively. In addition, as we know, the conductivity is the reciprocal of the resistivity. Therefore, the average thin film conductivity of the organic heterojunction film before and after ssDNA immobilization can be calculated to be $0.521 \times 10^{-3} \text{ S/cm}$ and $0.699 \times 10^{-3} \text{ S/cm}$.

Furthermore, since the performance of biosensor devices may be affected to some extent by the absorbed substances and the sensitivity of proposed heterojunction device is very important for potential applications. We systematically investigated the influence of the concentration of ssDNA solution on the electrical response of proposed device, as well as its' stability in air. Fig. 4 (a) shows the response of device with different ssDNA solution concentration, which was calculated by the current of the device at 50V according to Eq. 1. It was found that the response of device with PBS-only is negative (-6%) because solution permeation in organic materials is inevitable, and usually causes degradation in organic semiconductor devices [17]. Nevertheless, the response of the device gradually increases with the ssDNA concentration firstly, and then tends to be saturated when the concentration is higher than $30 \mu\text{g/ml}$.

Then, we studied the air stability of the device without and with ssDNA immobilization at a fixed concentration of 50 $\mu\text{g/ml}$. As seen in Fig. 4 (b), for the device without ssDNA, the current increased rapidly by 8% in the first few hours, then it decreases and tends to be stable after 33 hours. The reason for the first increase is due to the oxygen exposure, which could lower the carrier injection barrier [18]. When the oxygen adsorption is saturated, the device current becomes stable. The reason for the second slightly decrease may be attributed to the moisture absorption and permeation. On the other hand, devices with ssDNA immobilization were also measured. The device exhibited good air stability because the current only reduced by 2.7% after stored the sample in air for 140 hours. The negligible reduction can be also explained by the above moisture effect. Moreover, oxygen always act as electron acceptor, which in turn generates holes and causes the reduction of negative charges in DNA molecules [19].

Finally, the thickness dependence of CuPc upper layer on the sensitivity of $\text{F}_{16}\text{CuPc/CuPc}$ planar organic heterojunction device with ssDNA immobilization (50 $\mu\text{g/ml}$) has been studied in detail. First, we measured the I-V characteristics of the devices with different thickness of CuPc upper layer. The thickness of F_{16}CuPc is fixed at 15 nm, whereas the thickness of CuPc varies from 5 to 20 nm. As shown in Fig. 5(a), when the thickness of CuPc changes from 5 nm to 10 nm, there is a jump for current. The jump gradually reduces, and finally disappears when the thickness is larger than 15nm. The behavior can be explained by space charge accumulation. Owing to the strong charge transfer between n-type F_{16}CuPc and p-type CuPc, electrons accumulated in the F_{16}CuPc layer, and holes accumulated in the CuPc layer. The accumulation makes the carrier density increase remarkably in the two organic semiconductors, and the carrier density is strongly dependent on the thickness of each semiconductor layer. When the thickness of CuPc thin film increases, the holes in CuPc increase and a conductive path is forming for holes transport. Therefore, the current increases rapidly in the first several nanometers. But the hole density reduces exponentially from interface towards the surface. When the thickness of CuPc is larger than 15nm, the holes get saturated and the thickness of CuPc has little contributions to the total current transport. It should be mentioned that when the thickness of CuPc is greater than 15nm, the conductive behavior in CuPc can be separated

as conductive region and non-conductive region, as shown in Fig 6 (a). The non-conductive region is similar to intrinsic CuPc, which contains lots of defects and traps [20-22].

Then, we measured the electrical responses of the same devices with ssDNA immobilization. The currents under 50V for both type devices are plotted in Fig. 5(b). All devices exhibit increased current when ssDNA molecules are immobilized. By applying Eq. (1), the response of the devices can be evaluated, which is summarized in Table 1. The highest response of 109.4 % was obtained by using 5nm CuPc, which means device with 5nm CuPc has the highest sensitivity for ssDNA detection. The electrical response gradually decreases with the increasing of CuPc layer thickness and finally it becomes insignificant for device with 20 nm CuPc layer. Since the surface morphologies are similar, it is logical to expect that same positive charges can be induced near the surface of CuPc after DNA immobilization. However, the influence of DNA immobilization on conductivity of the organic heterojunction could be completely different as depicted in Fig. 6 (a) and Fig. 6(b). Fig. 6(a) represents a device with thick CuPc layer, such as 20 nm. As we have discussed, because of the saturation of holes, the DNA-induced positive charges only possess a small proportion and hence they have limited contribution to the whole current transport. At the same time, they can be easily captured by traps in the bulk of CuPc. Fig. 6(b) represents the case for device with thin CuPc layer such as 5 nm. The holes in thin CuPc layer are not saturated and the traps near CuPc surface are going down with reducing CuPc thickness because most of them have already been filled by holes induced by charge transfer. Thus, the induced positive charges have significant influence on the whole current transport.

4. Conclusion

In conclusion, we demonstrated an F₁₆CuPc/CuPc planar organic heterojunction device that could sense the ssDNA immobilized on CuPc surface. The accumulation of electron and hole at heterojunction interface can form a conductive channel, which is sensitive to the external influences. Our results proved that the planar organic heterojunction device have a sensitive electrical response to ssDNA immobilization. The AFM images showed that the CuPc surface has been well covered by the immobilization of ssDNA molecules, whose negative charge induced additional holes in CuPc

layer, resulting in the increase of device conductivity. The response and stability of the device was also disclosed. The response has a strong dependence on the concentration of solution that will gradually exhibit a saturation phenomenon with the increase of ssDNA concentration. And the conductivity of the devices increase rapidly in the first few hours, then it tends to become stable after exposing them in air for about two weeks. Furthermore, the electrical response of device with ssDNA immobilization can be improved by reducing the thickness of the upper CuPc layer because the ssDNA-induced holes in different area of CuPc have different impact on the whole conductivity of the device. We believe the above results are helpful for the development of planar organic heterojunction based sensors. Further work will be done on the immobilization of double-strand DNA and DNA hybridization based on planar organic heterojunction devices.

Acknowledgements

Wen Gu gratefully acknowledge the research start-up funding from Shanghai University of Engineering Science, Shanghai Municipal Education Commission under Grant No. ZZgcd14009, and National Science Foundation for Young Scholars of China (No. 61404082).

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Fig. 1. The electrical characteristics of the organic heterojunction devices (a) before and (b) after ssDNA immobilization (channel length is 30 μm).

Fig. 2. The AFM morphology of CuPc thin films (a) before and (b) after ssDNA immobilization.

The insets are the amplifying AFM images.

Fig. 3. (a) The current and (b) total resistance of devices under different channel length before and after ssDNA immobilization (current was measured at 50 V).

Fig. 4. (a) Electrical response of the planar organic heterojunction devices for different ssDNA concentration; (b) Air stability test of the devices with and without ssDNA immobilization.

Fig. 5. (a) The thickness dependence of CuPc upper layer on the electrical characteristics of $F_{16}CuPc/CuPc$ heterojunction devices and (b) on the current response by ssDNA immobilization (current was measured at 50 V, channel length is $30\mu m$).

Fig. 6. Schematic diagram of carrier distribution in ssDNA-induced $F_{16}CuPc/CuPc$ heterojunction

interface with (a) thick $CuPc$ and (b) thin $CuPc$ upper layer.

Table 1. Summary of the electrical response on organic heterojunction devices with ssDNA immobilization.

CuPc thickness (nm)	5	10	15	20
$I_o(\mu A)$	0.772	1.915	2.334	2.404
$I_{DNA}(\mu A)$	1.617	2.844	3.016	2.642
Response (%)	109.4	48.5	29.2	9.9

Dear Editor Enrique Calleja,

Thank you very much for your letter and the comments from the referees about our paper submitted to Solid State Electronics (Ms. Ref. No.: SSE-D-16-00219).

We very much appreciate the careful reading of our manuscript and valuable suggestions of the reviewers. We have checked the manuscript and revised it according to the comments. Note that the red font sections in the manuscript have been modified.

If you have any question about this paper, please don't hesitate to let me know.

Sincerely yours,

Dr. Gu

Reviewers' comments:

I appreciate the effort on behalf of the authors to improve the discussion on how the ssDNA adsorption could affect the conductivity of the heterojunction, as well as the sensitivity of the device as a sensor (pages 9-10 of the manuscript), by including a new figure as well as some clarifying text. Thus, my objections to this point have been removed.

With respect to my first recommendation regarding the comparison of the sensitivity of this heterojunction device with that achieved by other systems (based, for instance, on OFETs), *my objections still remain*. Sensitivity is a quantitative parameter and must be compared in a quantitative (and not qualitatively) way. The so-called Table 1 in the author's answer is qualitative and not acceptable. In the sensors field, performance of many materials and devices may be affected to some extent by the absorbed substances, so it is necessary to provide a quantitative comparison to assess the relevance of a new proposal. In this case, this is a manageable challenge, because performance of alternative sensor devices for the ssDNA have been reported (those based on pentacene) and accurately described in the literature. Even in case where authors would consider the results of this comparison unfavorable, they can discuss why in the text, as done in the author's

answer.

In my opinion, only after this 2nd point was properly addressed, the paper may be accepted for publication.

Our answer:

According to the reviewer's comments, our answers are as follows:

First, we are very sorry to make some mistakes about the concept of sensitivity.

Action: The sensitivity in Table 1 and most part of the text is replaced by another noun named electrical response.

Second, the reviewer think performance of many materials and devices may be affected to some extent by the absorbed substances, so it is necessary to provide a quantitative analysis.

Action: We have carefully revised the paper and supplemented the influence of ssDNA concentration on the electrical response of the proposed devices. Please see page 6 and figure 4(a).

Third, the reviewer is interested in the comparison of the sensitivity of the proposed device with other systems (for example OFETs).

Explanation: As we know, the sensitivity of the two systems (our proposal device and OFET) is affected by many factors, such as organic semiconductor materials, thickness, thin-film surface morphology and so on. Besides, the sensitivity of OFET is also dependent on the value of gate voltage. Usually, lower gate voltage results in higher sensitivity. Hence, it is very hard to quantitatively compare them.

Action: We have revised the paper and deleted all the description of OFET in the text to avoid the conflict between them. Moreover, we added an experiment about the influence of DNA concentration on the performance of device. The results indicated that the lowest ssDNA concentration of 0.5 μ g/ml can be well detected.

Thanks to the careful reading and valuable suggestions of the reviewer.